Strategies for the detection of food pathogens and contaminants

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Strategies for the detection of food pathogens and contaminants

Biacore Food Analysis Symposium 2006
Kempinski Hotel, Berlin, September 27th 2006

Stephen Hearty, *Paul Leonard, Alfredo Darmanin Sheehan, Sharon Stapleton, Elizabeth Tully, Lynsey Dunne, Stephen Daly, Barry McDonnell, Peter Skottrup and Richard O'Kennedy*

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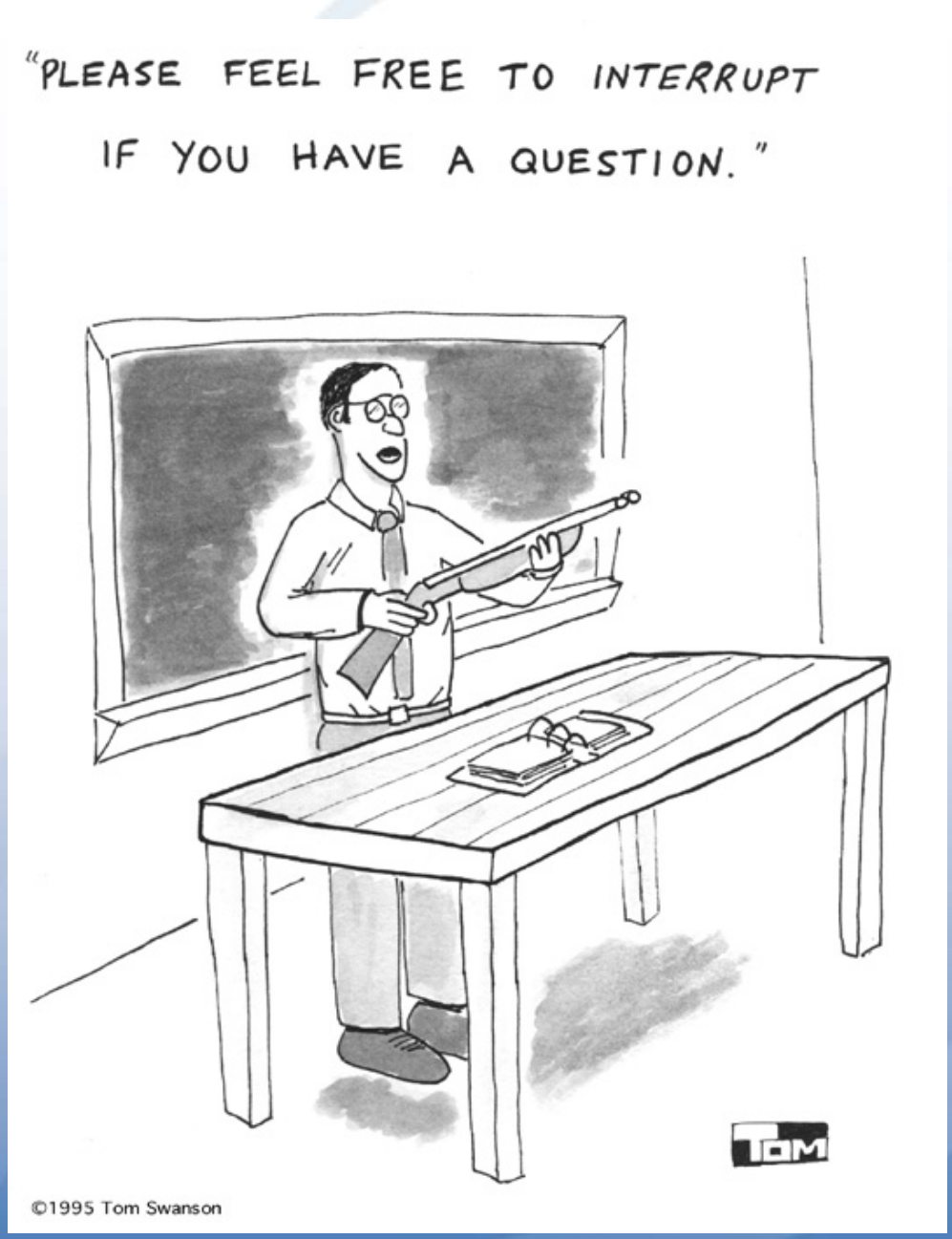
"PLEASE FEEL FREE TO INTERRUPT
IF YOU HAVE A QUESTION."
TOM
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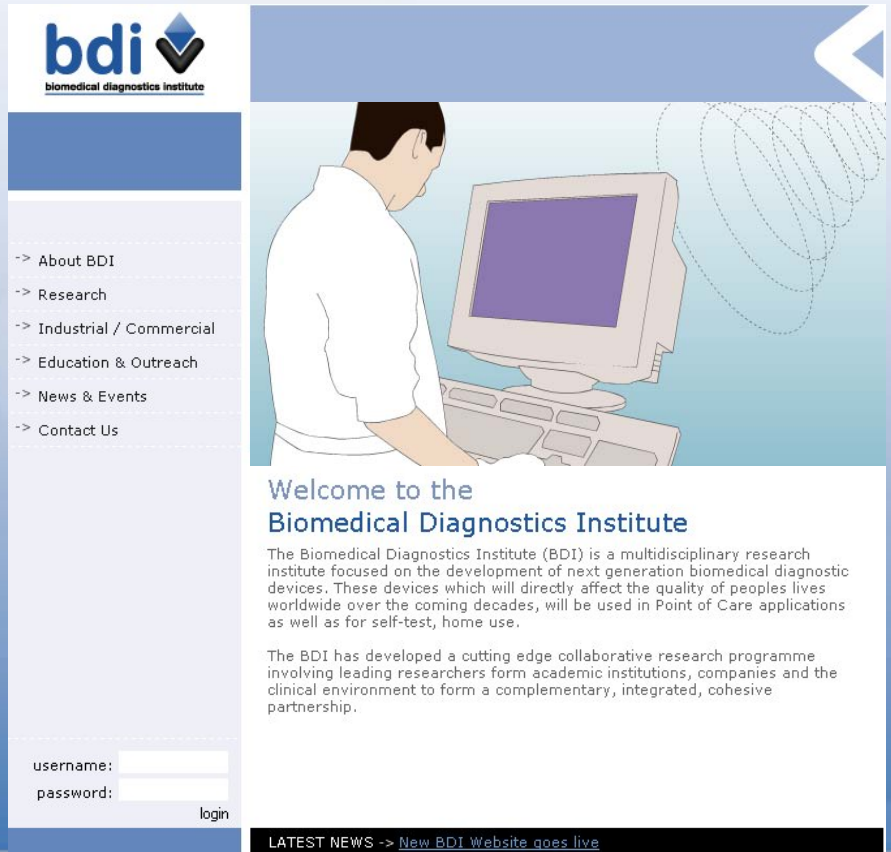
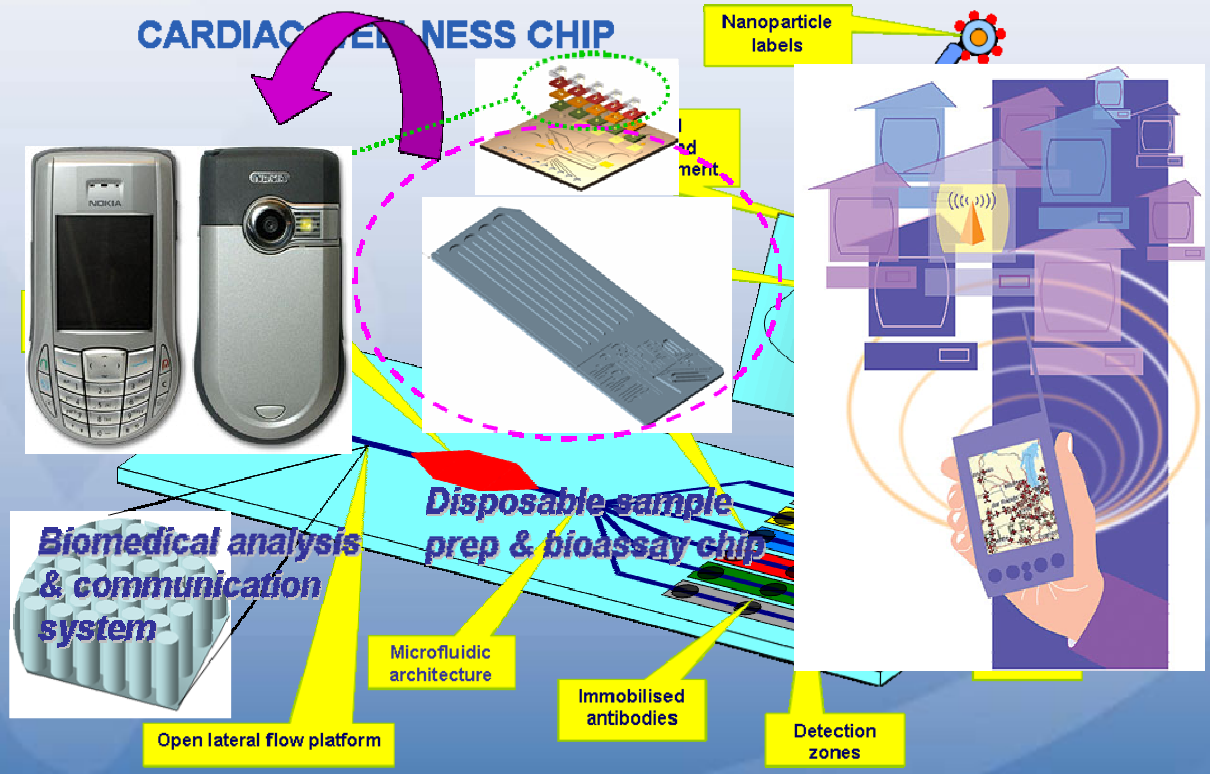
Biomedical Diagnostics Institute

- Established 1st Oct. 2005
- SFI CSET: Industry-Academic Partnership
- Investment: SFI €16.5m, Industry €6m



Biomedical Diagnostics Institute

- **Next-generation Biomedical Diagnostics**
  - **Early warning of life-threatening events**
  - **Link therapy and monitoring**
  - **POC and self testing (home use)**



Biorecognition Group

…what do we do?

"….the production, optimisation and characterisation of antibodies and antibody fragments".

Overview

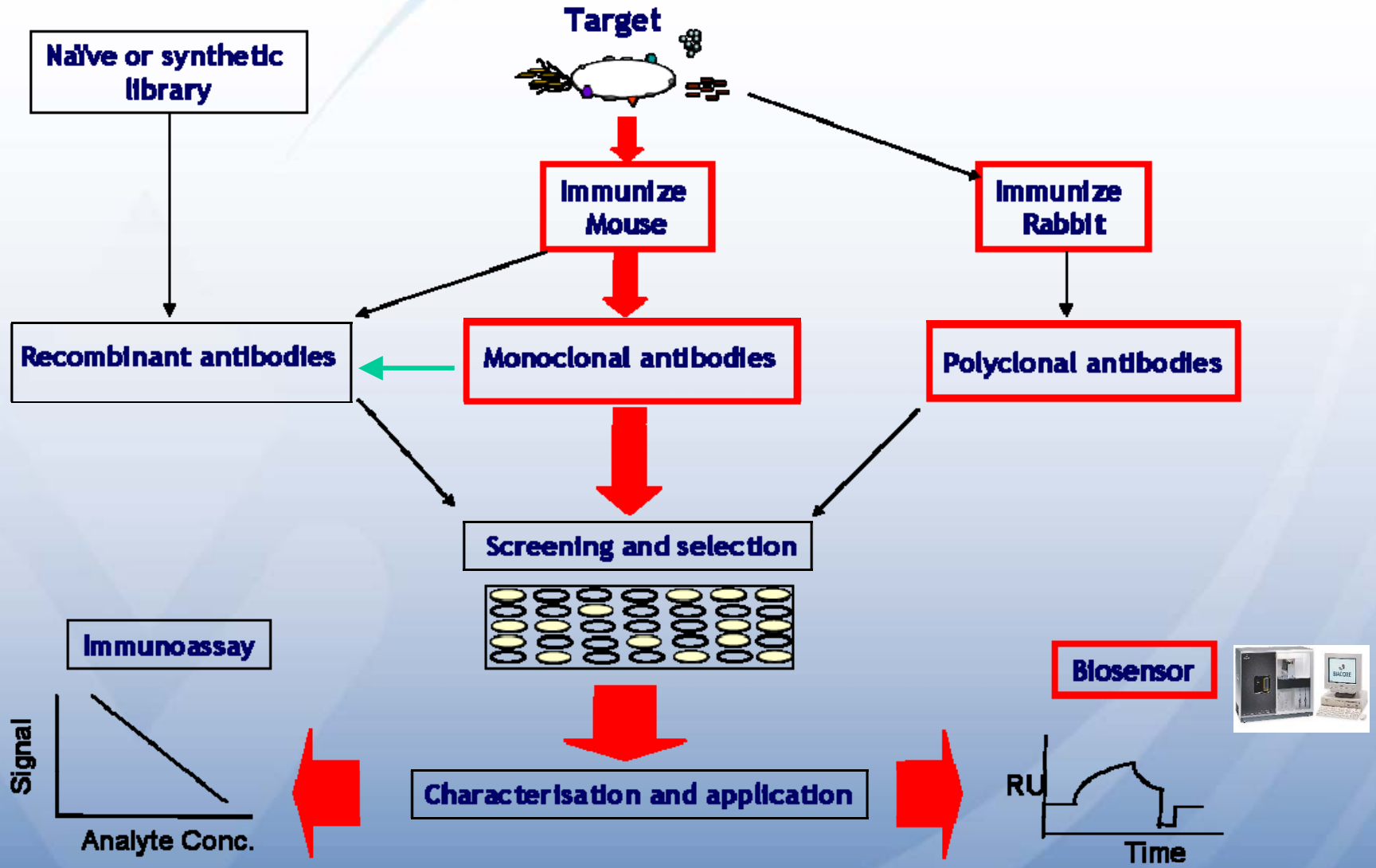
- ***Listeria monocytogenes*** (Whole cells)
  - Subtractive inhibition assay (anti-whole cell pAb)
  - Direct inhibition assay (anti-InlB pAb)
  - Direct cell binding (anti-InlA mAb)

- ***Puccinia striiformis*** (Spores)
  - Subtractive inhibition assay (anti-spore cell mAb)

- **Aflatoxin B1**
  - Inhibition assay (anti-AFB1 scFv)

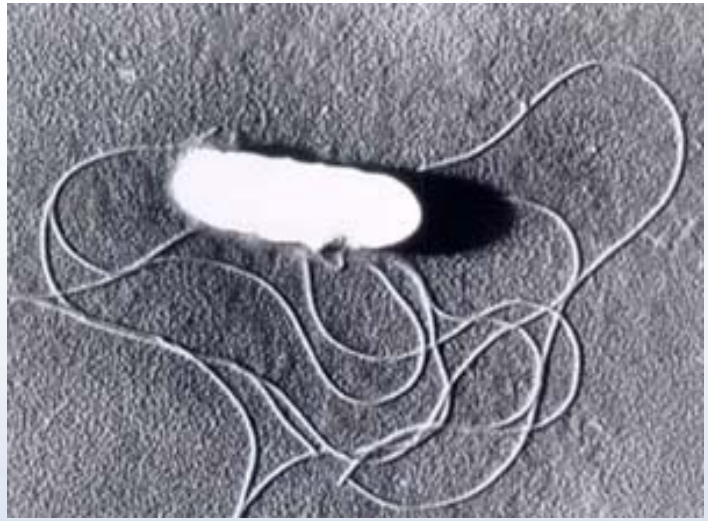
- **Cephalexin**
  - Inhibition assay in milk (mutated scFv antibody)

Strategy


Target
Naïve or synthetic library
Immunize Mouse
Immunize Rabbit
Recombinant antibodies
Monoclonal antibodies
Polyclonal antibodies
Screening and selection
Immunoassay
Signal
Analyte Conc.
Characterisation and application
Biosensor
RU
Time

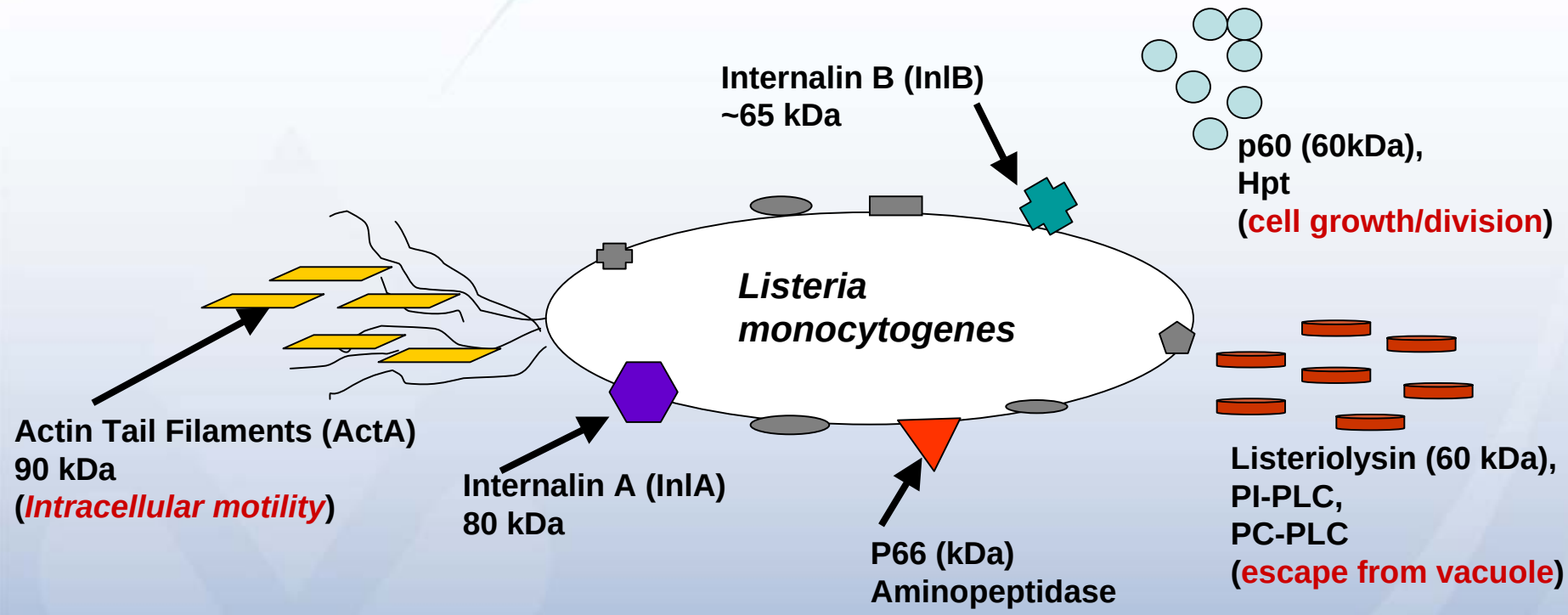
Listeria monocytogenes

- Gram positive bacterium
- Ubiquitous in nature
- Grow at 4^{o}C
- ‘Listeriosis’ manifested as
  - food poisoning
  - spontaneous abortion
  - meningitis and encephalitis
  - >20% mortality rate

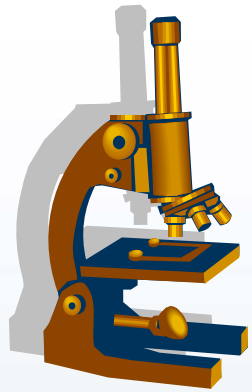


http://textbookofbacteriology.net/Listeria.html

Virulence Proteins



Current Detection



First stage enrichment (24-48 hours)

↓

Second stage enrichment (24-48 hours)

↓

Verification (24 hours)

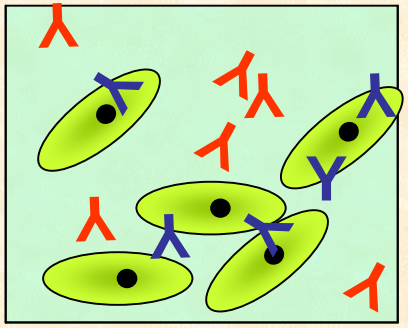
.....***too long***!!

Subtractive inhibition

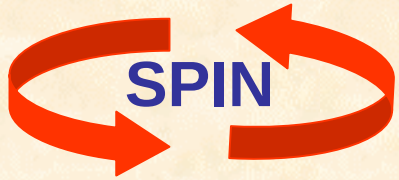
- Polyclonal antibody produced against whole cells
- Biacore assay developed:
  - Assay format
  - Preconcentration
  - Immobilisation
  - Specificity
  - Regeneration
  - Assay validation

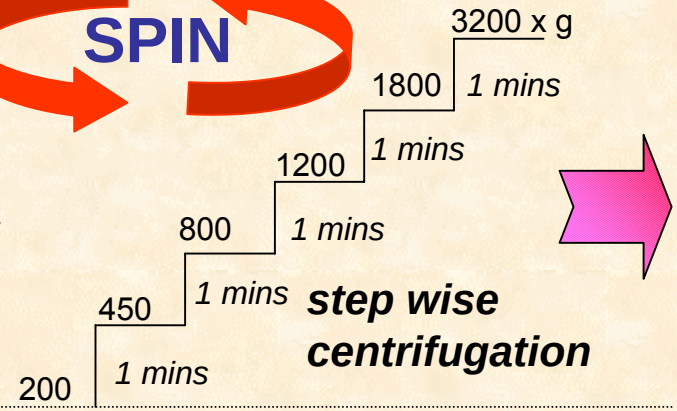
Assay: subtractive inhibition

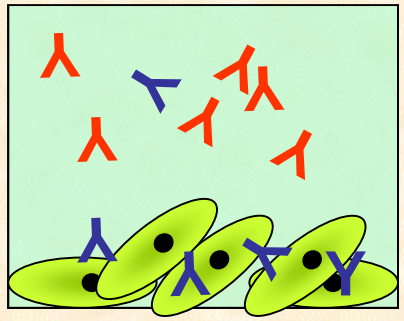
Sample prep.



20 min incubation



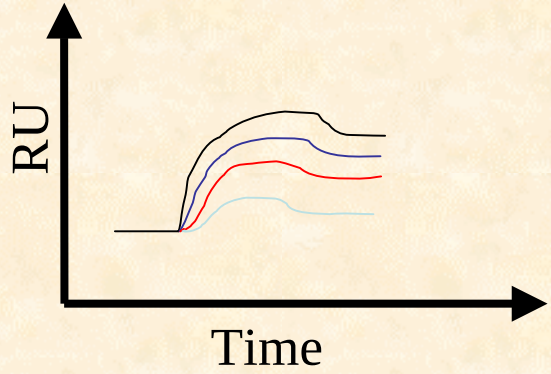


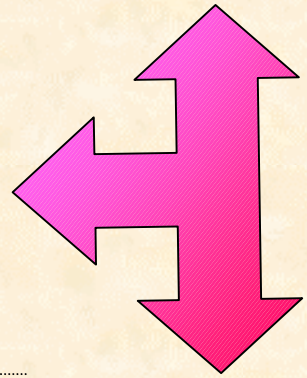


Keep supernatant

Output

Sensogram of decreasing free antibody concentrations



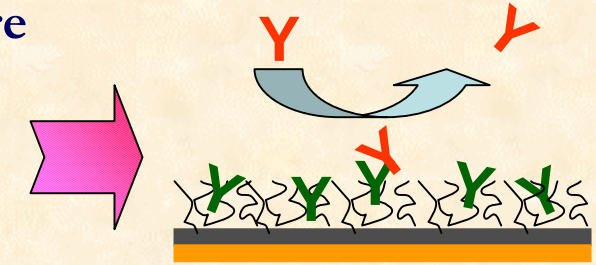


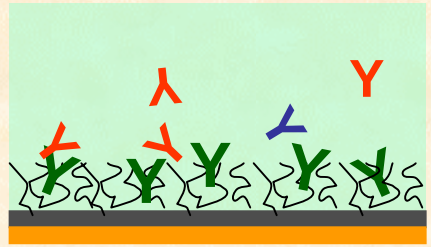
Add antibody supernatant

CM5 chip prep.

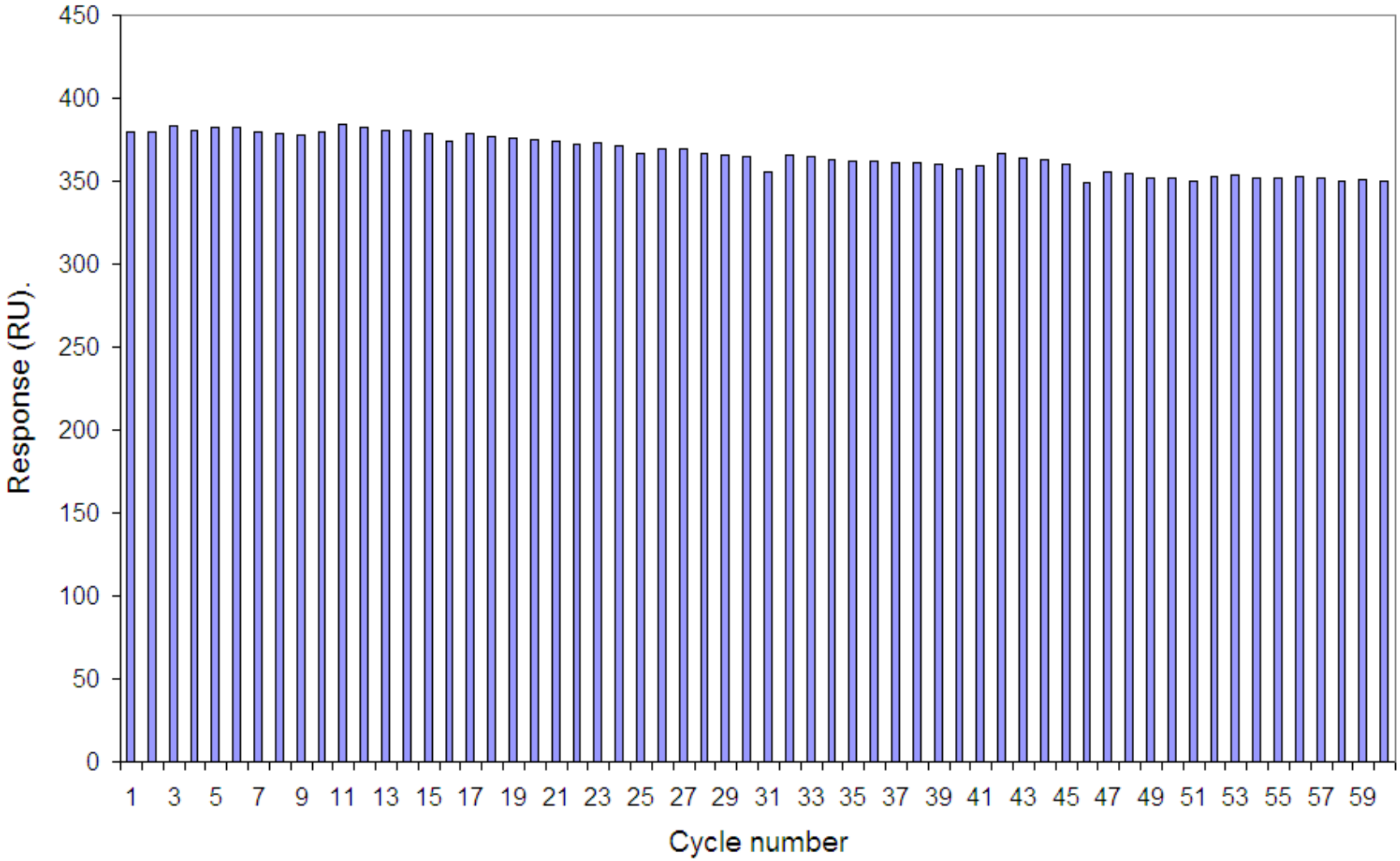
Immobilise capture antibody (Y)

Optimise regeneration

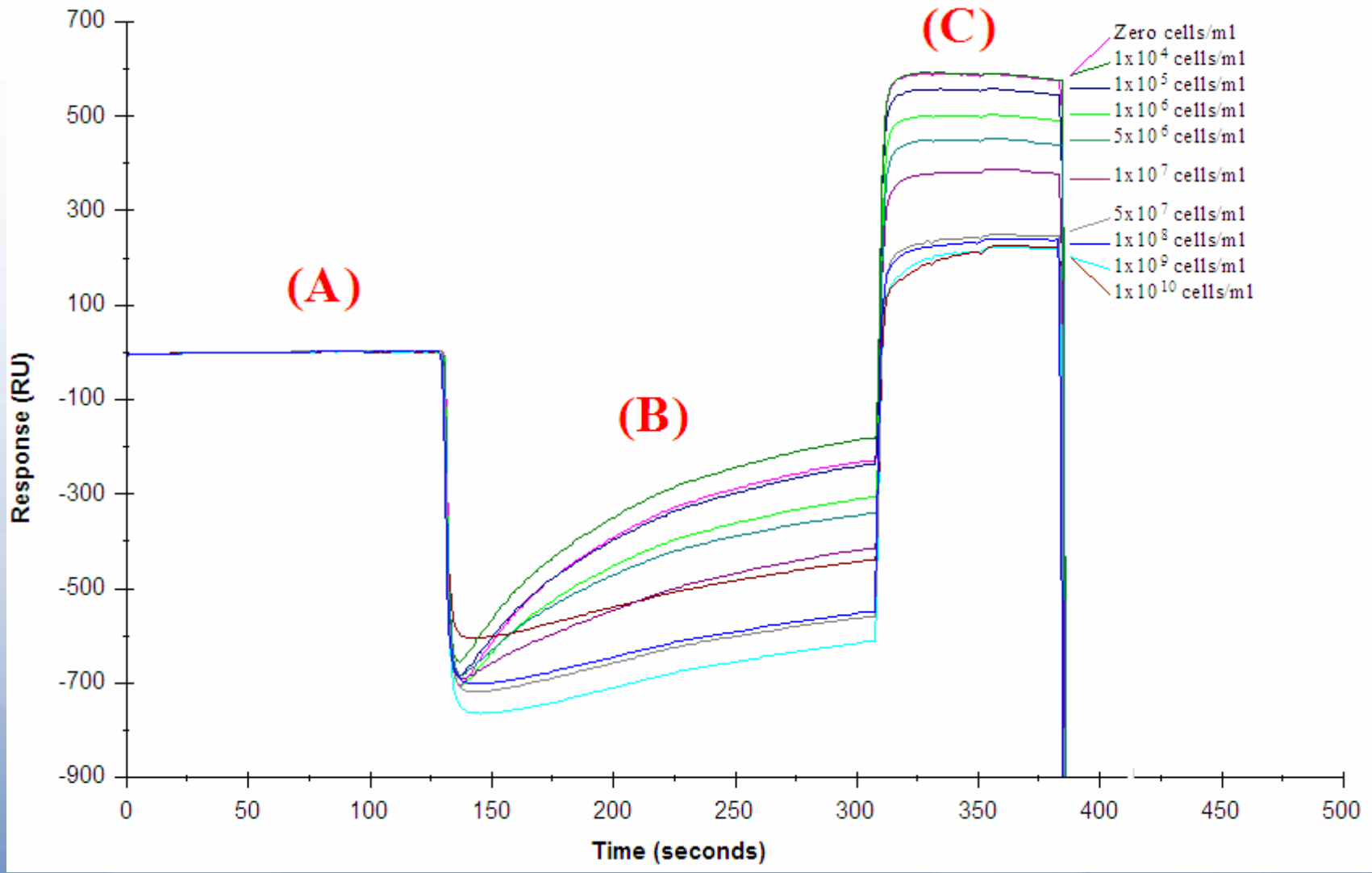




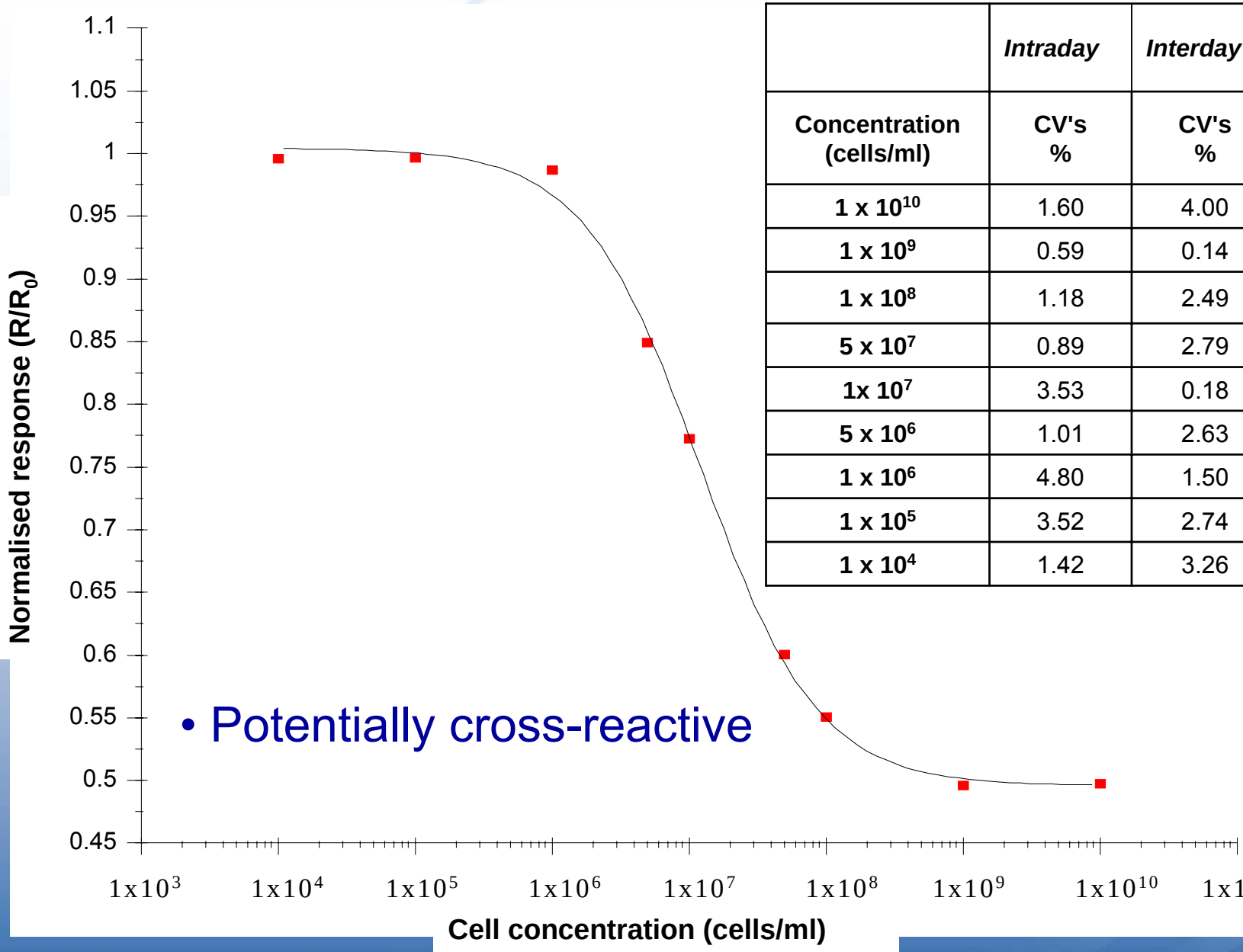
Regeneration



Overlay Plot



Typical Calibration Curve

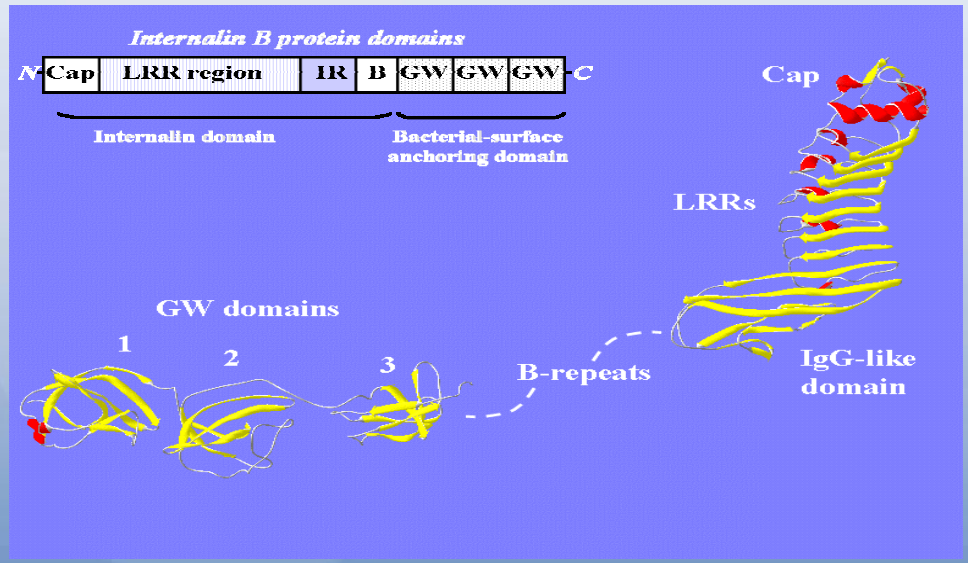


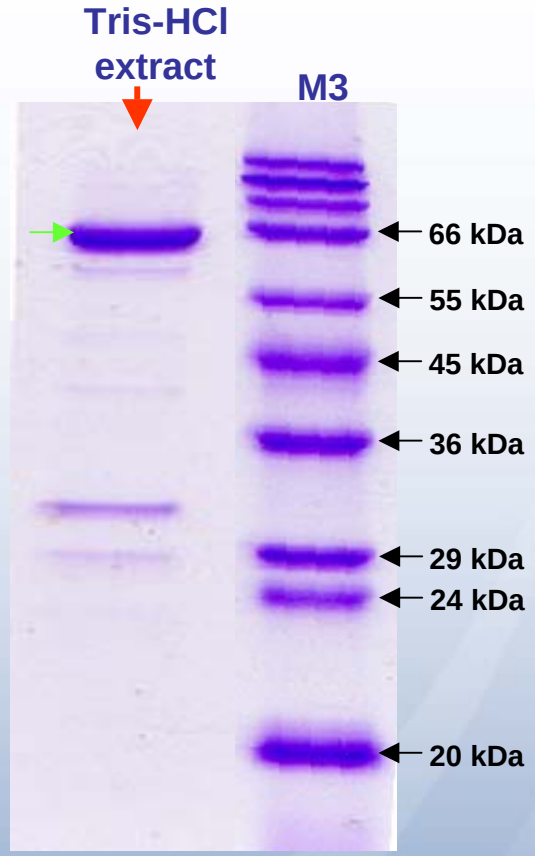
	Intraday	*Interday*
Concentration (cells/ml)	**CV's %**	**CV's %**
1 x 10^{10}	1.60	4.00
1 x 10^{9}	0.59	0.14
1 x 10^{8}	1.18	2.49
5 x 10^{7}	0.89	2.79
1x 10^{7}	3.53	0.18
5 x 10^{6}	1.01	2.63
1 x 10^{6}	4.80	1.50
1 x 10^{5}	3.52	2.74
1 x 10^{4}	1.42	3.26

- Potentially cross-reactive

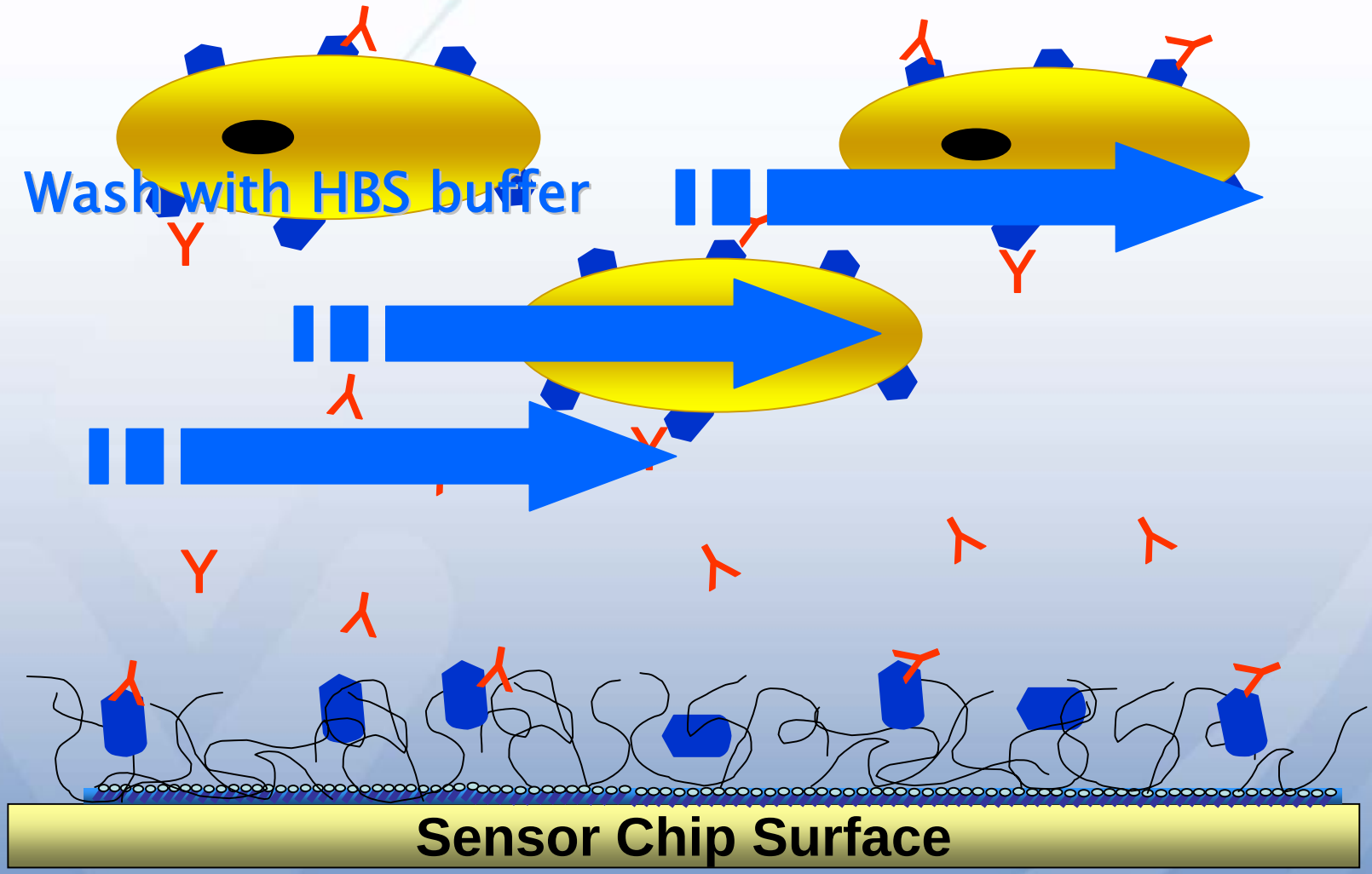
Direct Inhibition Assay

- Refine the specificity
- Anti-InlB extract polyclonal antibody
- Cloned InlB gene sequence
- Purified protein by IMAC

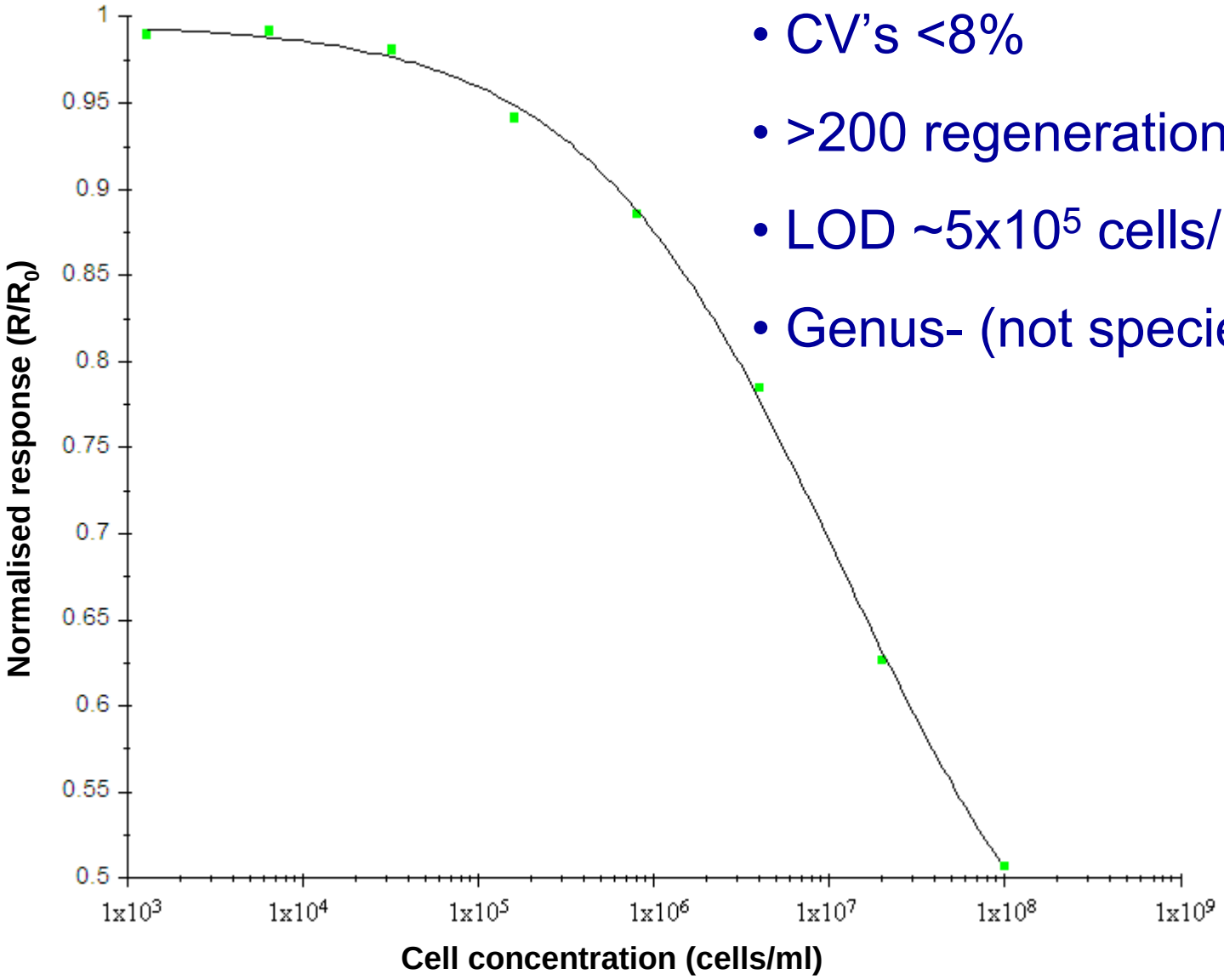




Inhibition Assay



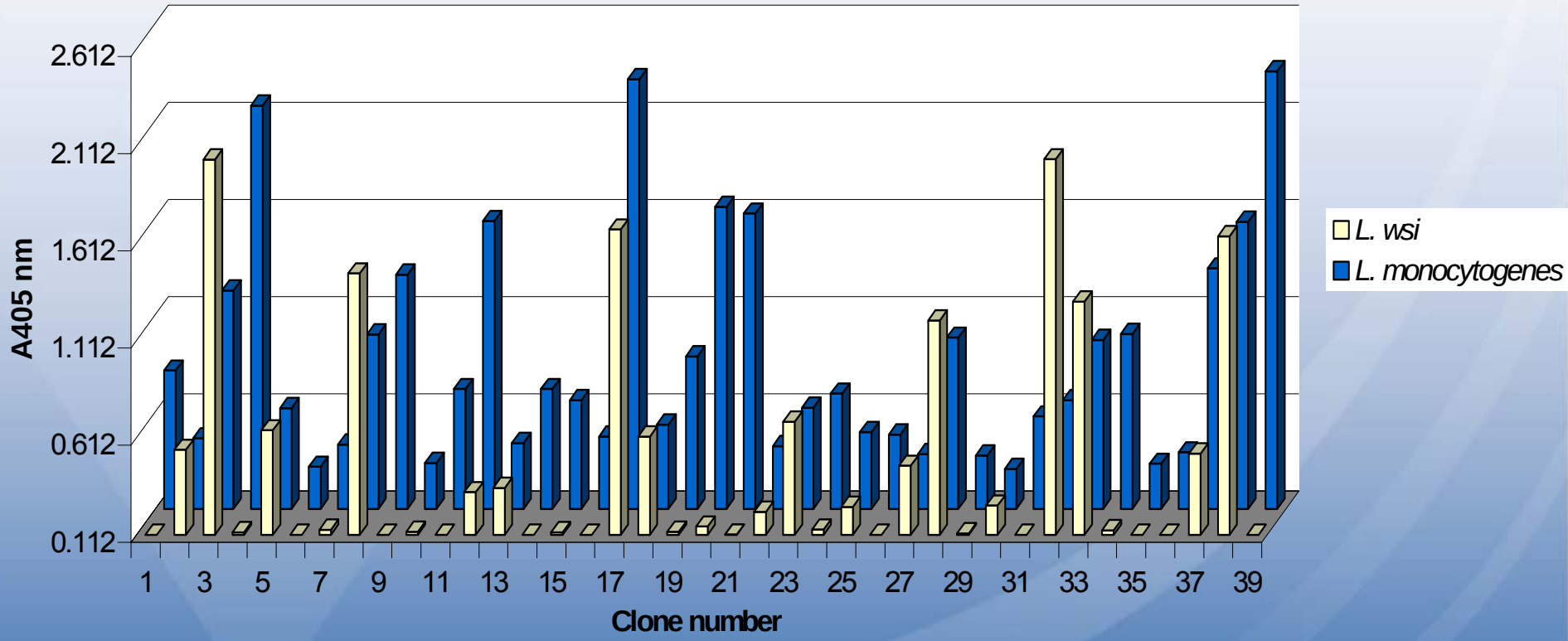
Calibration Curve



- CV's <8%
- >200 regeneration cycles
- LOD ~5x10^5 cells/ml
- Genus- (not species) specific

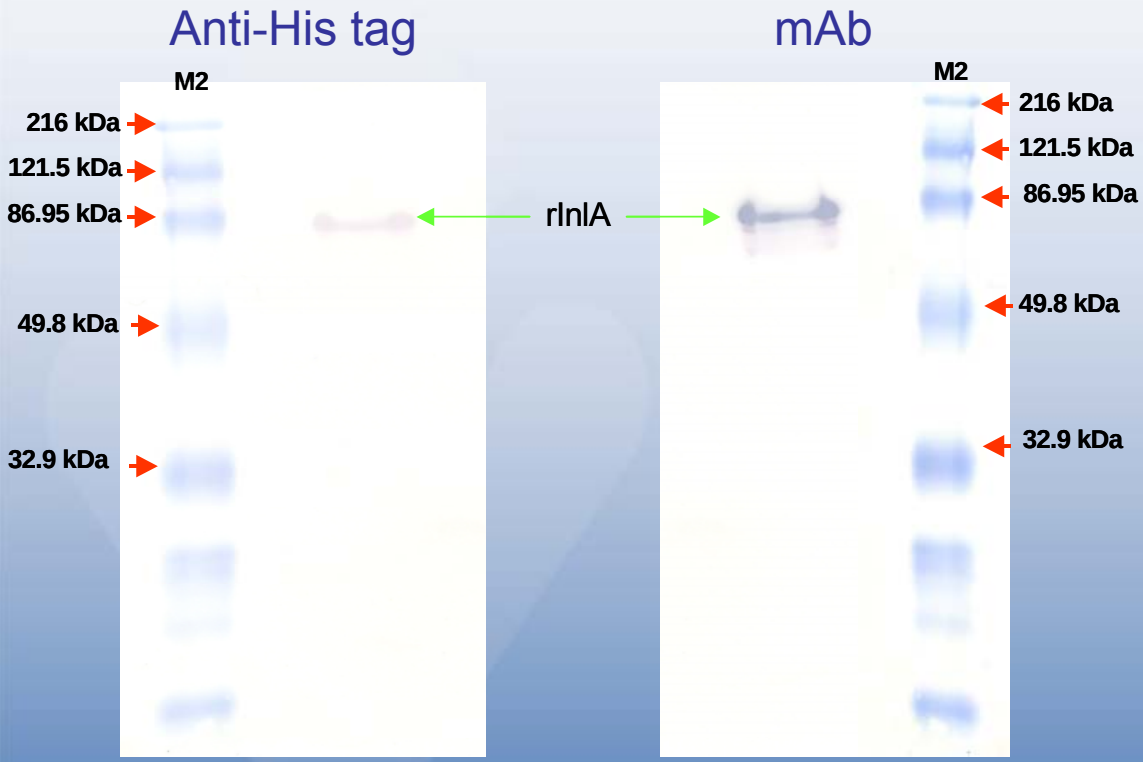
Whole Cell Immunisation

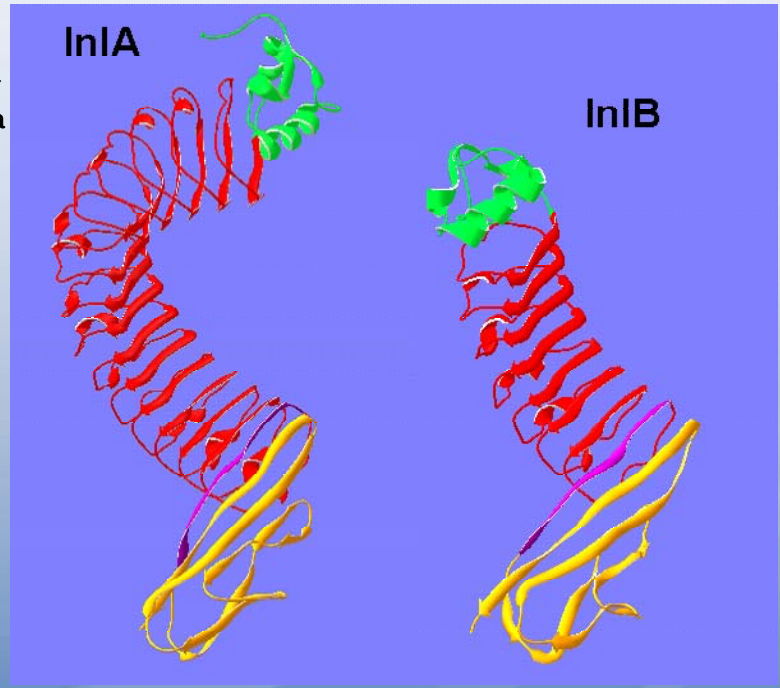
- Tandem (negative) screening
  - Putative *L. monocytogenes*-specific hybridomas
  - Selected clone no. 39 for further analysis



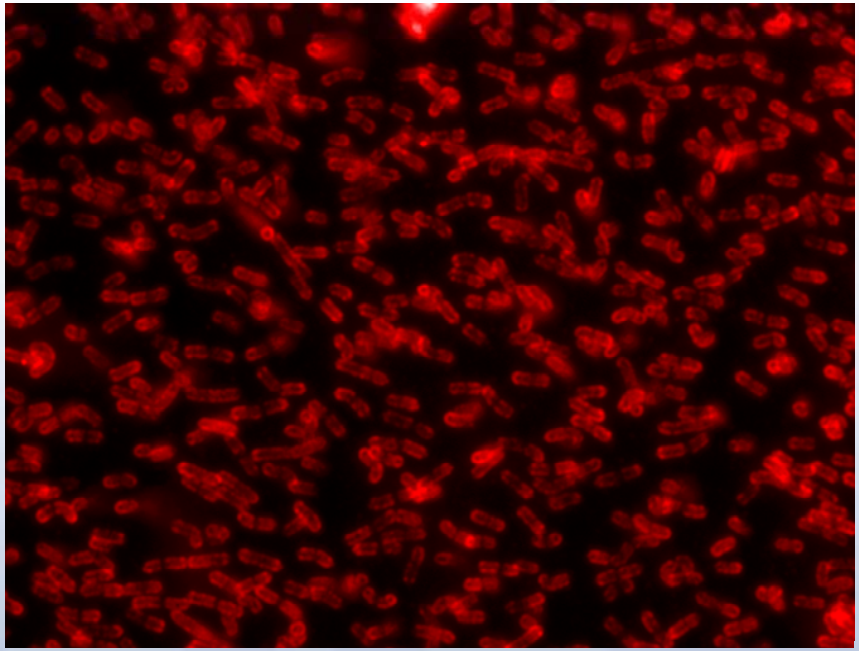
Epitope Mapping

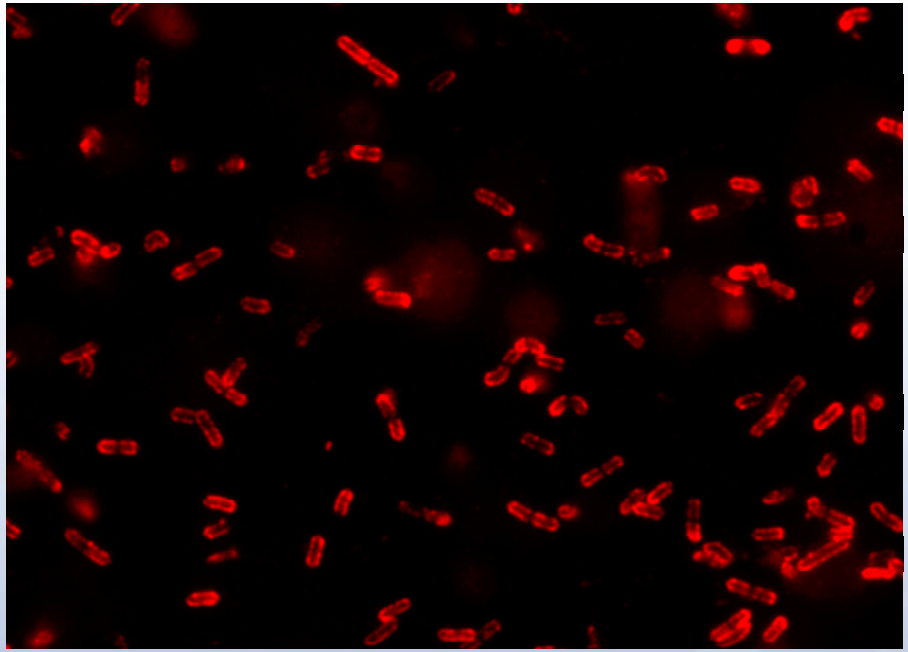
- Confirmed specific binding of mAb to native and rInlA protein
  - Internalin A (InlA)
  - Constitutively expressed key virulence determinanant





Immuno-staining

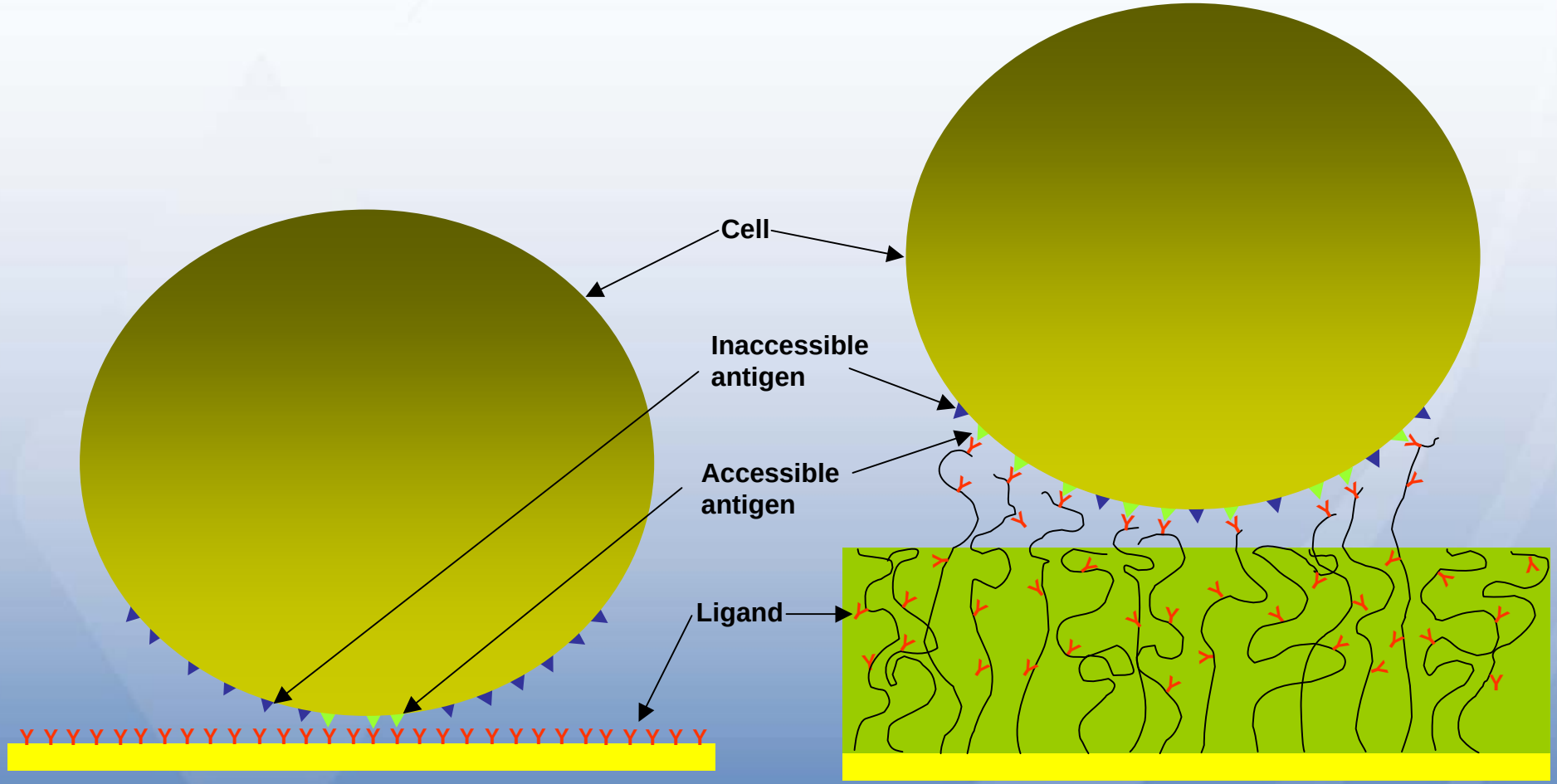




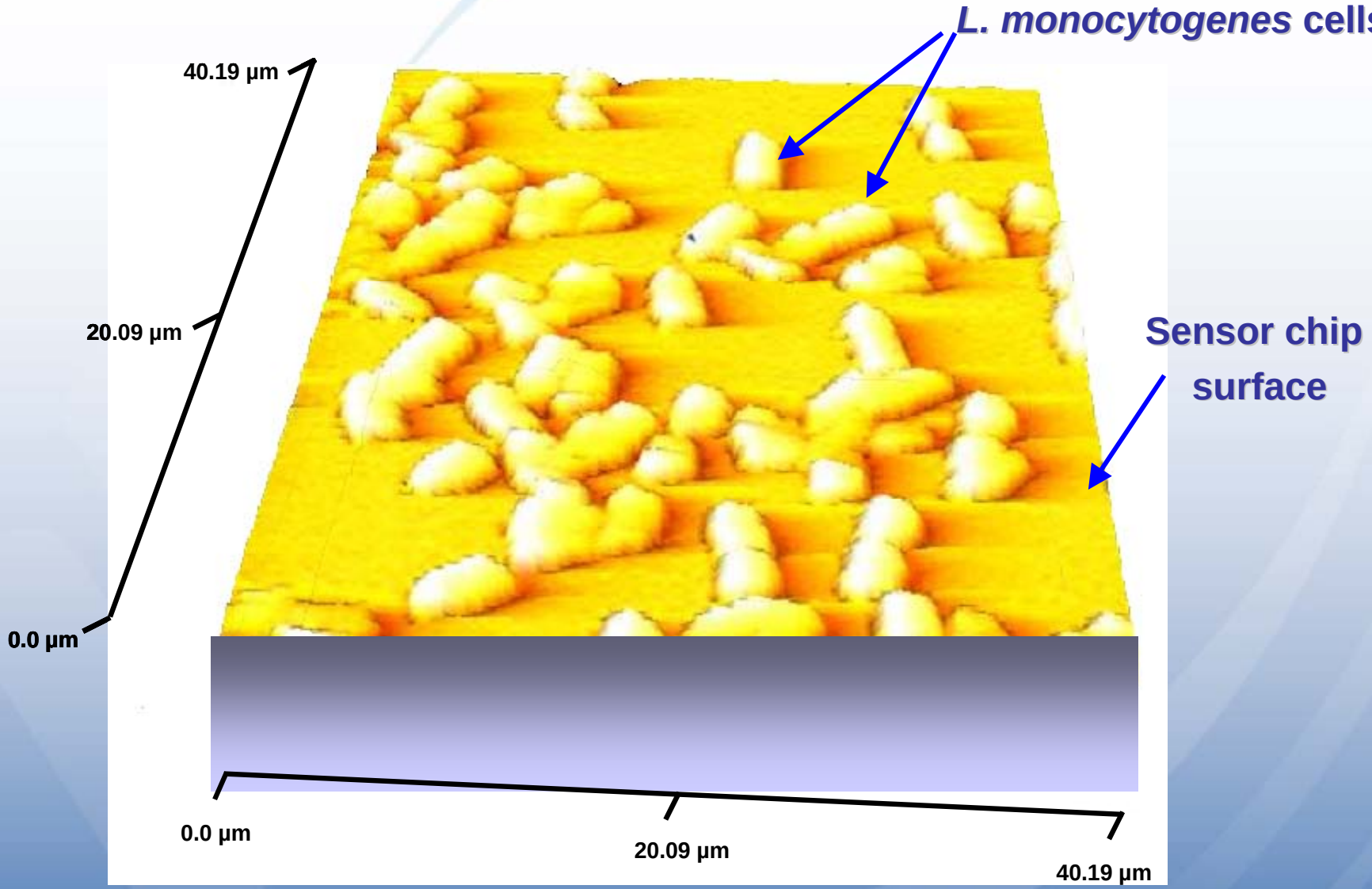
- **Use of anti-InlA antibody-linked red light-emitting quantum dots (605nm), for the visualisation of *L. monocytogenes* cells on poly-l-lysine treated glass slides.**

Choice of Sensor Chip

- Cell binding model

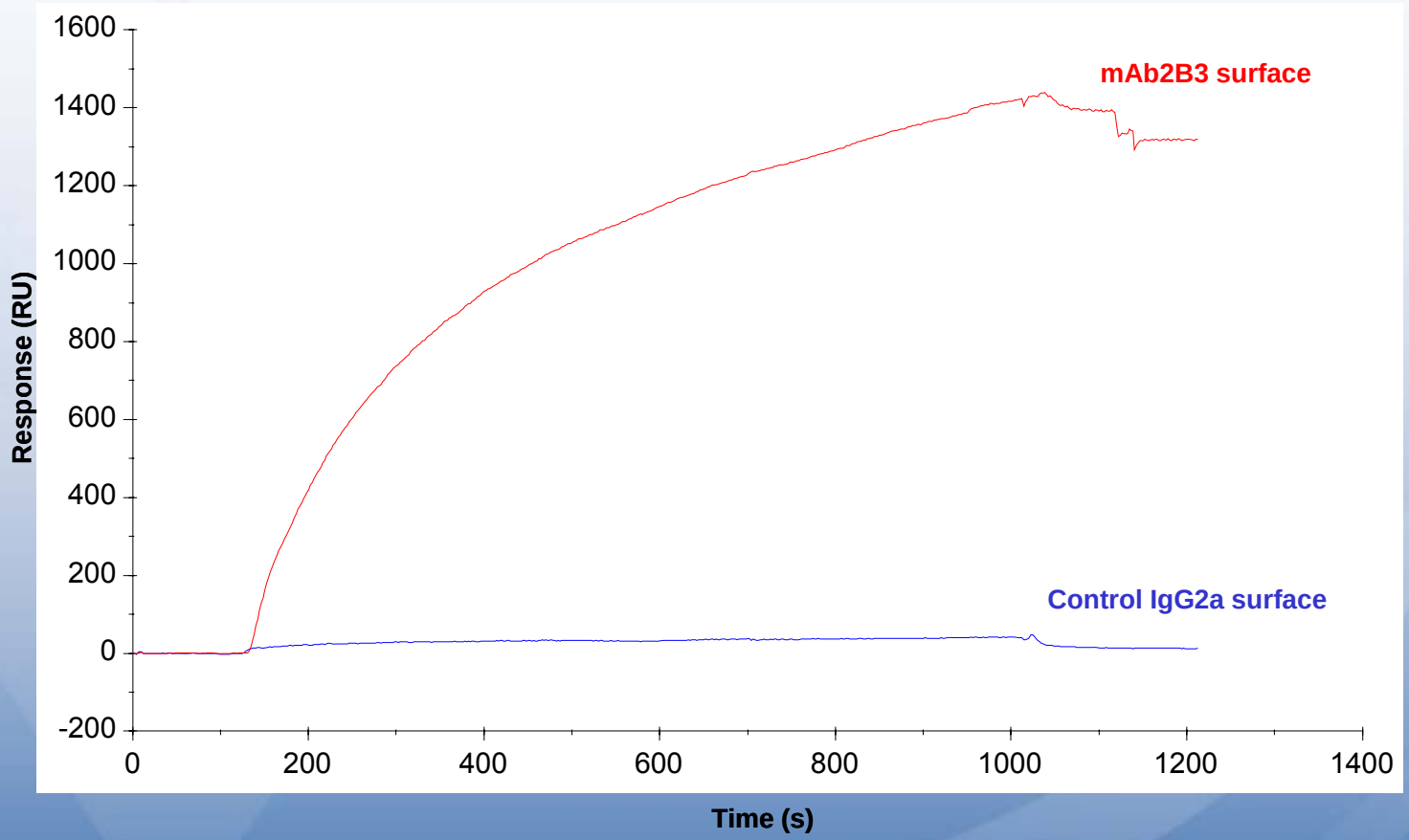


AFM Imaging


L. monocytogenes cells
Sensor chip surface
40.19 µm
20.09 µm
0.0 µm
0.0 µm
20.09 µm
40.19 µm

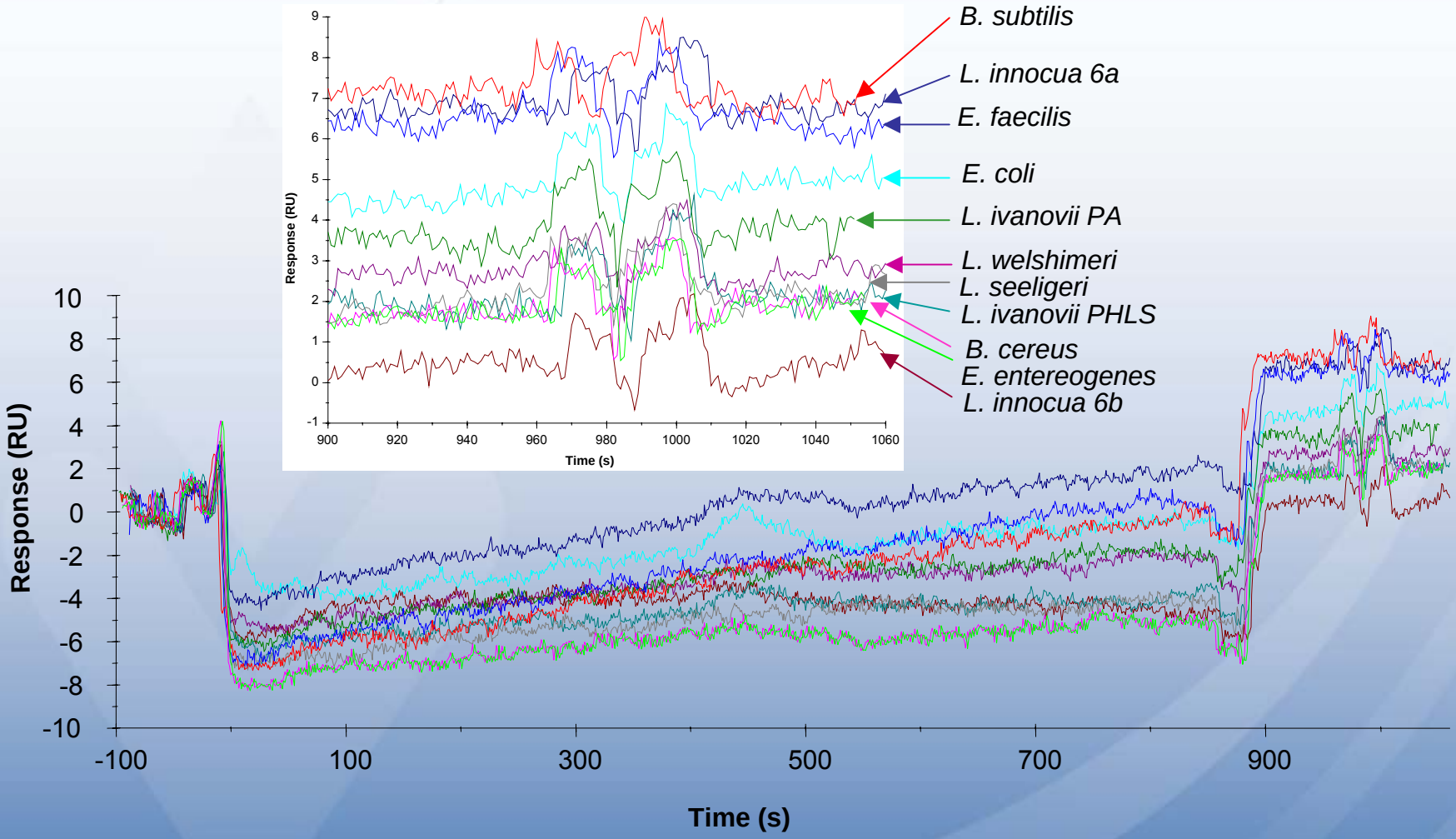
Surface Specificity

- Specific binding of *L. monocytogenes* cells to mAb immobilised surface

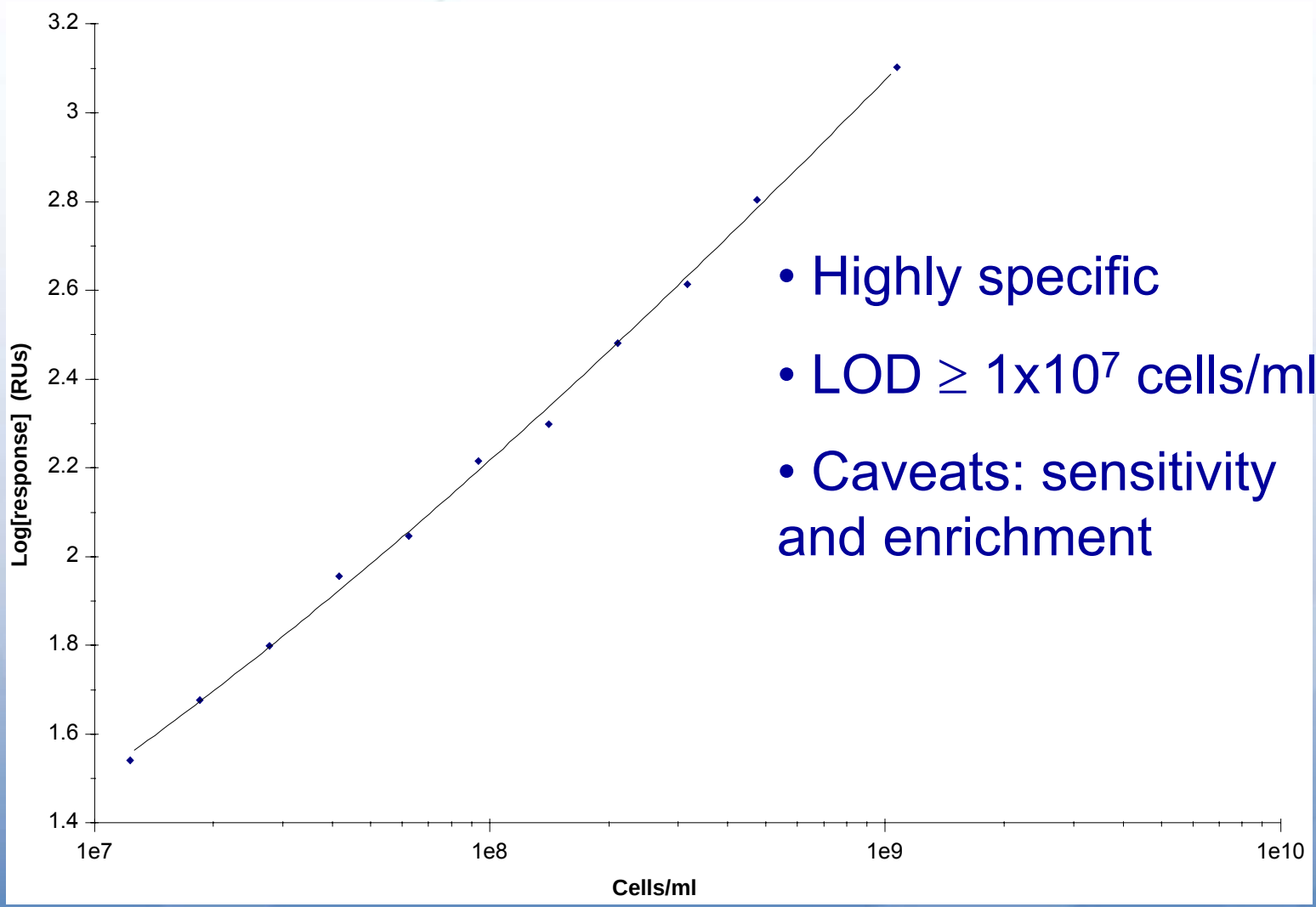


Surface Specificity

- No cross-reactivity



Typical Calibration Curve



- Highly specific
- LOD $\geq 1x10^7$ cells/ml
- Caveats: sensitivity and enrichment

Cereal Crop Screening

Macro-scale cereal crop farming

⇒ demand pre-harvest screening

"Precision farming"

↓

Mapping of disease areas

↓

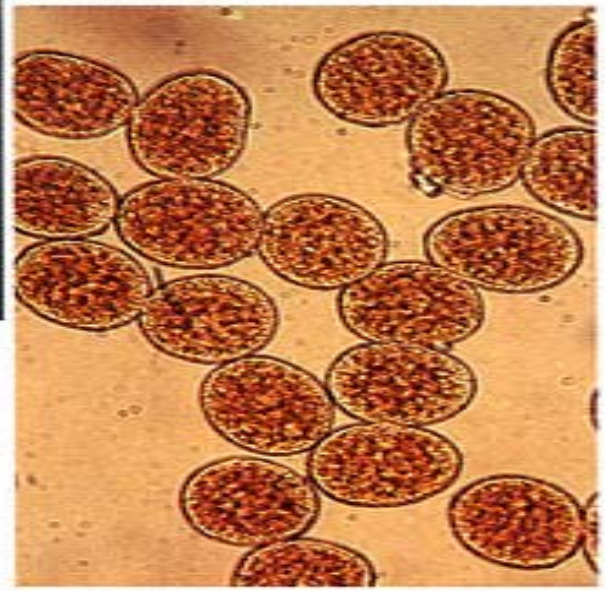
Targeted application of fungicide

Puccinia striiformis

STRIPE RUST

Uredinia

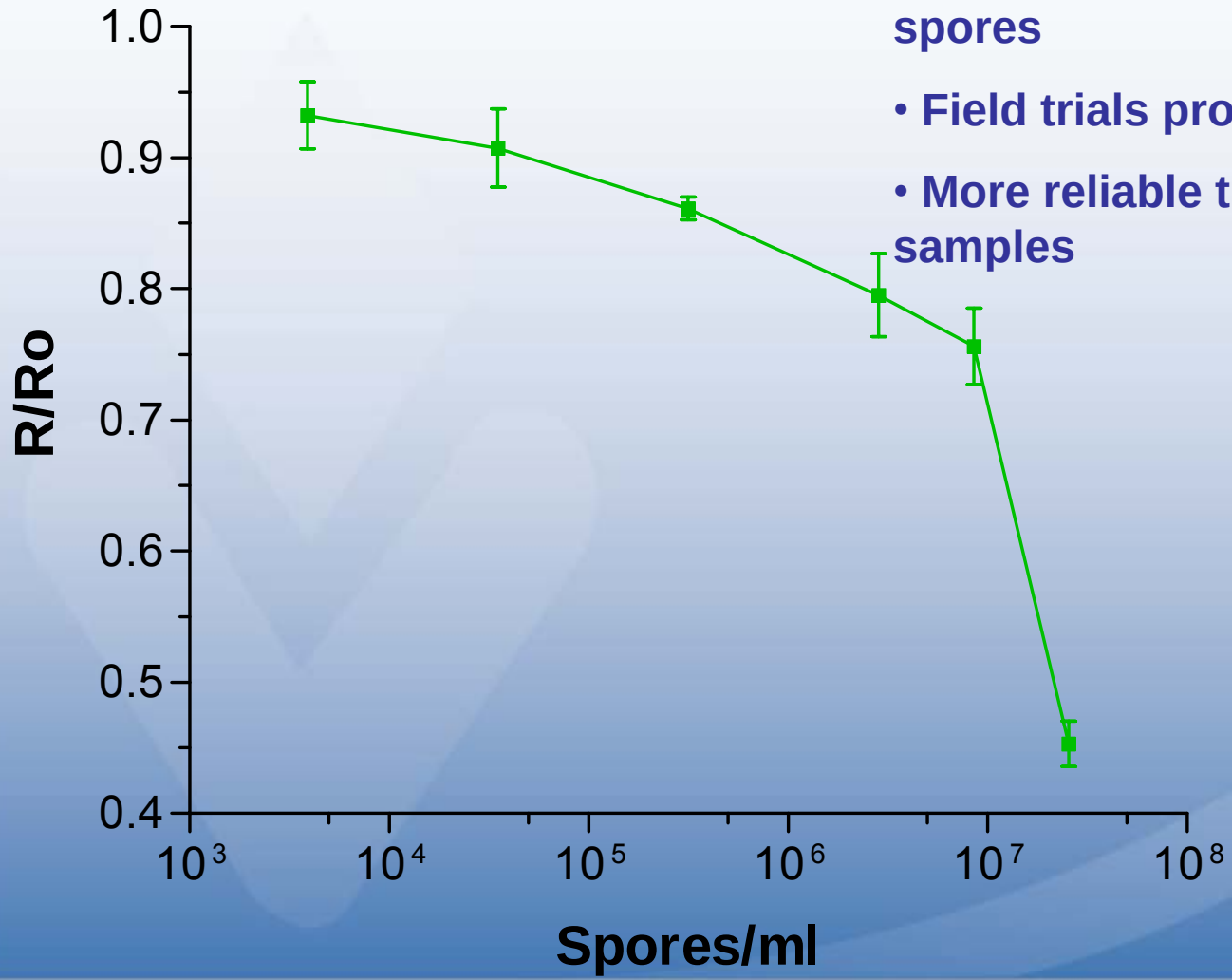
- 18-45 µm diameter



Urediniospores (400x)

Spore Calibration Curve

- Subtractive inhibition assay
- LOD: $3x10^5$ spores/ml
- First biosensor assay reported for *Pst* spores
- Field trials proposed
- More reliable than visual analysis of leaf samples



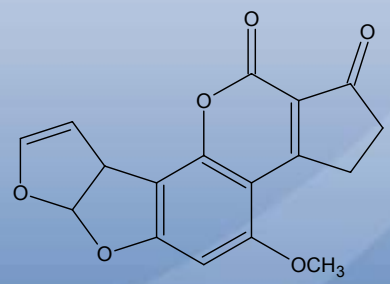
Aflatoxins

- A group of approx. 20 related fungal metabolites that occur in *Aspergillus* species

- Most common group of toxins from naturally occuring moulds

- Aflatoxin B1 (AFB1), produced by *A. flavus* and *A. parasiticus,* is the most predominant and toxic

- AFB1 is linked to human hepatocellular cancer



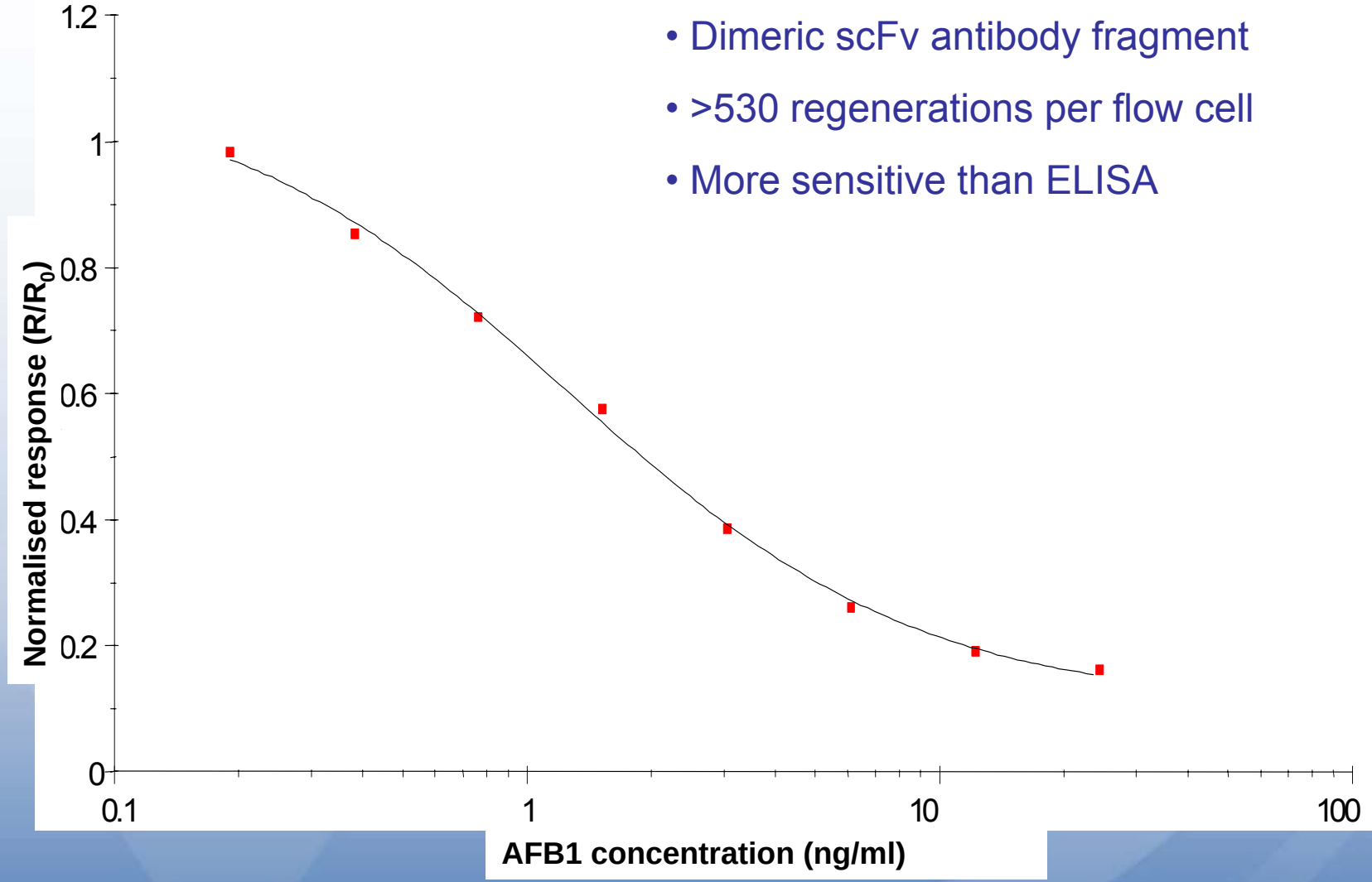
AFB_1 - 312 Da

Aflatoxins

- High humidity favours fungal growth and the production of aflatoxins
- Contamination is widespread in agricultural commodities
  - ⇒ **demand for post-harvest screening**
- Acute aflatoxin poisoning in man extremely unlikely and in animals rare
- Link between dietary exposure and liver cell cancer
- EU has set maximum residue limits for aflatoxins in a variety of food types (2-8ng/ml for AFB1)

Calibration Curve for Aflatoxin B1

- Dimeric scFv antibody fragment
- >530 regenerations per flow cell
- More sensitive than ELISA



Biacore and ELISA IC_{90} comparison

Anti-AFB_1 antibody	Competitive ELISA	Biacore inhibition assay	Improvement
Monomeric scFv	12ng/ml	0.39ng/ml	30 fold
Dimeric scFv	3ng/ml	0.19ng/ml	15 fold
Bifunctional scFv	3ng/ml	N/A	N/A

- Biacore can significantly out-perform ELISA

Cross-reactivity

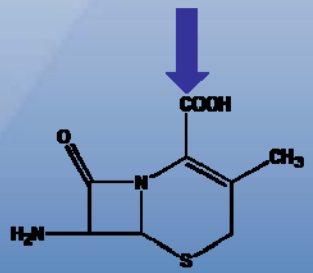
Aflatoxin	CR_{90} (%)	CR_{50} (%)
B_1	100	100
B_2	<1	1.2
M_1	1	1.5
M_2	<1	<1
G_1	10	20
G_2	<1	1.2

The cross reactivity potential was approximated at the least detectable dose (LDD), which is estimated at 90% R/R_0, and at the IC50 value, which is estimated at 50% R/R_0. The CR90 and CR50 were then expressed as 100-fold the ratio of the antigen and of the cross-reactant.

Cephalexin

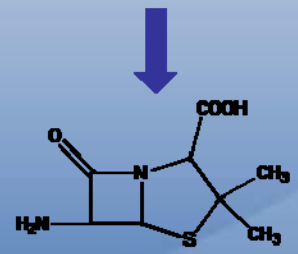
- Cephalexin is a member of the cephalosporin sub-family of antibiotics.
- Characteristic four membered β-lactam (2-azetidinone) ring
- Antibiotics used to treat livestock (e.g. Mastitis)
- Associated with an increase in body mass (i.e. sub-therapeutic dosage)
- Public health (e.g. MRSA, allergic response) and economic concerns for producers and processors

Cephalosporin Based β-lactams



7-Amindodesacetocephalosporanic Acid

Penicillin Based β-lactams

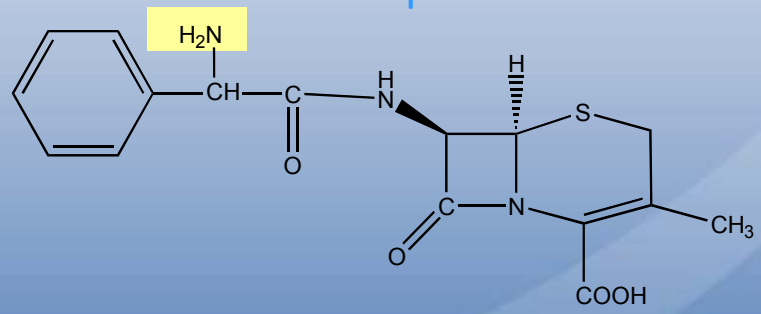


(+)-6-Aminopenicillanic Acid

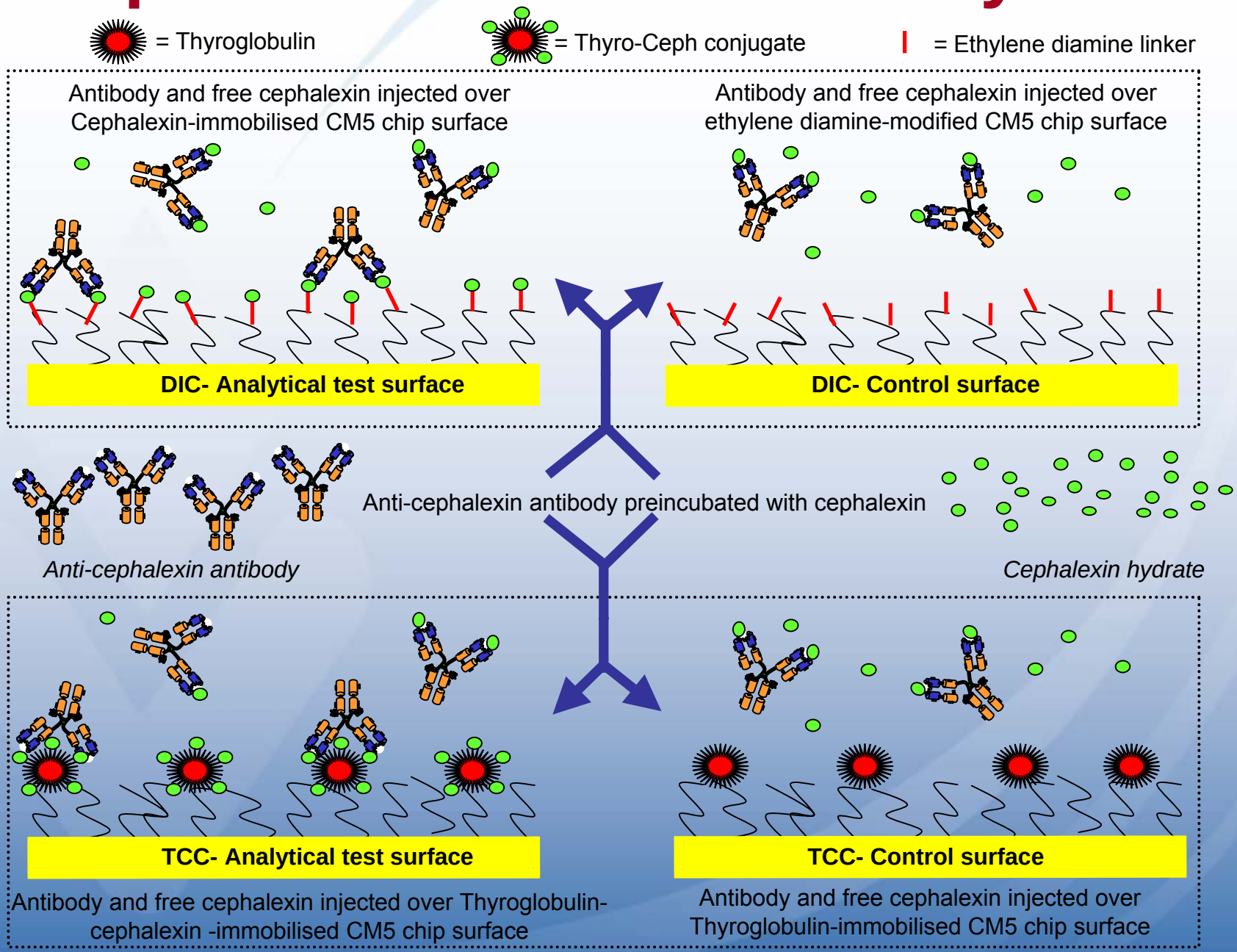
Current detection

- Standard rapid assays for antimicrobial detection are based on microbial inhibition or bacterial receptor assays (e.g. Delvo SP, charm test)
- May be followed by a confirmation test (e.g. HPLC)
- Manufacturers quote limits of detection (LOD's) of 40-60 ng/ml for the Delvo SP for cephalexin
- EU Legislation
- MRL: 100 ng/ml

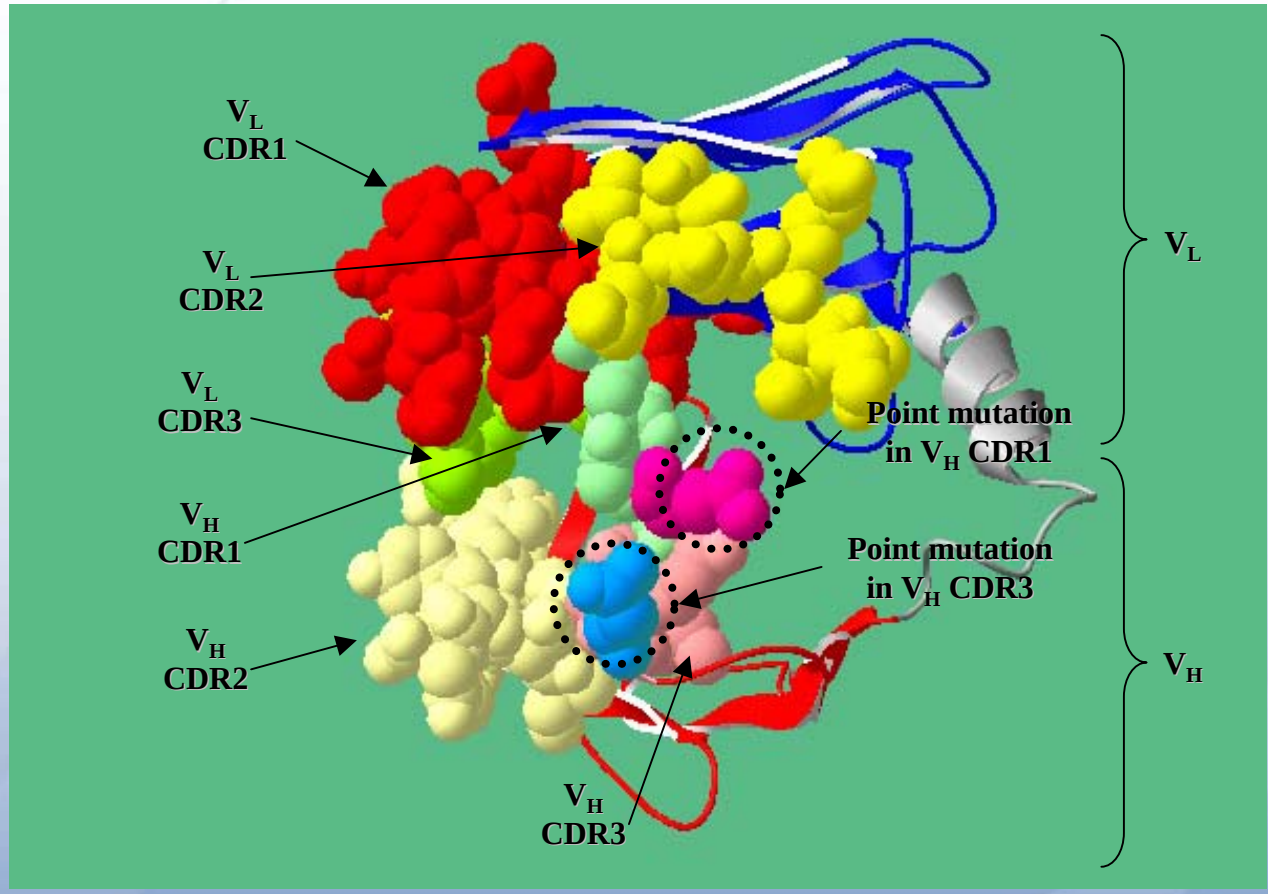
Cephalexin



Cephalexin Inhibition Assay Format



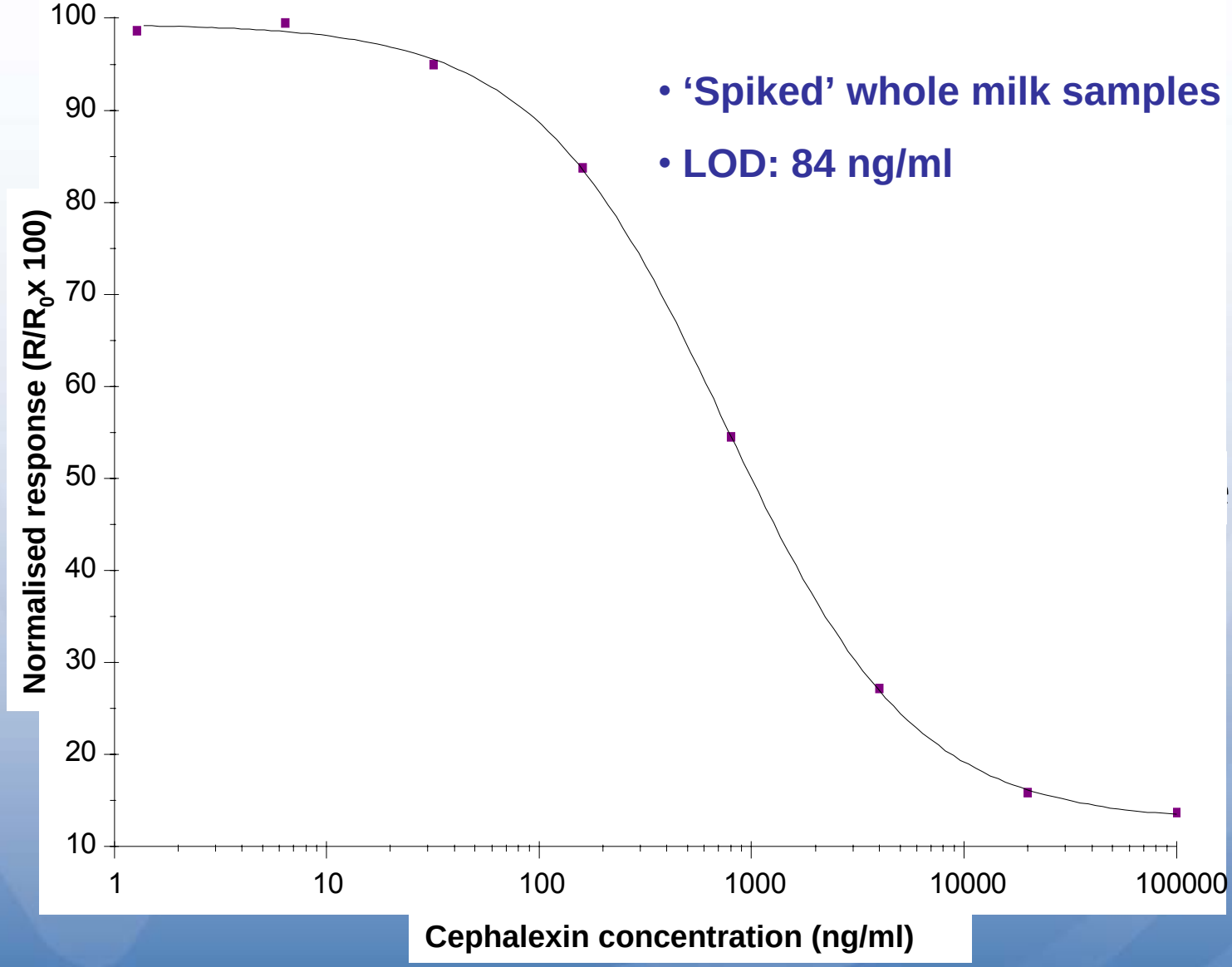
Anti-Cephalexin scFv



CDR H3 WT- G L G Y G K A F M D Y
C5 - G Q G Y G K A F M D Y

CDR H1 WT- S Y G M S
C5 - N Y G M S

Calibration Curve for Cephalexin



- ‘Spiked’ whole milk samples
- LOD: 84 ng/ml

Cephalexin Assay Validation

Response $RU/RU_0*100 \pm$ S.D.	Concentration of Cephalexin (ng/ml)	Back Calculated value (ng/ml)	% CV's	% Accuracies
13.68 ± 0.45	100,000	76,214.2	3.28	131
15.86 ± 0.94	20,000	22,256.1	5.94	90
27.14 ± 0.67	4,000	3,956.4	2.47	101
54.47 ± 0.05	800	801.5	0.10	100
83.69 ± 1.85	160	159.2	2.21	101
95.89 ± 3.21	32	33.4	3.35	96
99.43 ± 0.81	6.4	---	0.81	---
98.67 ± 3.45	1.3	5.9	2.03	22

Presentation summary

- Three *L. monocytogenes* immunoassay formats presented
- Monoclonal antibody produced that is highly specific for *L. monocytogenes*
- *Pst* spore detection demonstrated
- Biacore assay developed for the detection of Aflatoxin B1
- Aflatoxin B1 Biacore assay proved more sensitive than ELISA
- Development of a Biacore-based assay for Cephalexin using randomly mutated scFv

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Questions

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